Hb Hope: A Rare Variant of Haemoglobin-Forged a Rise in HbA1c on Immunoturbidimetric Assay

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Abstract Glycosylated hemoglobin (HbA1c) determination is a powerful means for assessing the evaluation and management of patients with diabetes mellitus. Hemoglobin (Hb) variants and chemically modified derivatives of Hb can affect the accuracy of measurement of HbA1c done by various analytical methods. We report a patient with a rare variant of Hb (Hb Hope) that caused an abnormally high value of HbA1c when assayed using immunoturbidimetric assay (“Tina-quant” 2nd generation assay) and also elucidate the nature of the variant.

Keywords Glycosylated hemoglobin · Diabetes mellitus · Immunoturbidimetric assay

Introduction

Long term glycemic control can decrease the morbidity and mortality associated with diabetes mellitus (DM). The regular measurement of HbA1c has been established as a valuable tool in the assessment of long term metabolic control in patients with DM [1].

A carbohydrate-protein linkage on the N terminus of β chains of hemoglobin (Hb), predominantly HbA in adults is called as the glycohemoglobin. HbA1c specifically refers to the majority of glycated species present. The initial reaction is the formation of an unstable aldimine that occurs between the carbonyl group of glucose and the N-terminal valine of the Hb β chain. The aldimine

undergoes Amadori rearrangement resulting in the formation of a stable Schiff’s base [2]. In healthy individuals, values of HbA1c reflect the degree of glycemic control over the preceding 2–3 months, reflecting the average lifespan of 120 days for RBCs.

A large number of clinical assays are commercially available for the determination of HbA1c. Most commonly used methods are affinity chromatography, ion exchange high performance liquid chromatography (HPLC) and immunoassays. These methods measure HbA1c based on its physical, chemical or antibody recognised characteristics. All of them can report falsely low value in conditions of diminished red cell survival. Structural variants and chemical derivatives of Hb interfere with many methods. The prevalence of variant Hbs have been demonstrated to be as high as one-third of all diabetics undergoing testing. In addition to genetic variants, structural modification of Hb that results from chemical alterations are often present in diabetic patients. Inaccurate measurements of HbA1c are especially problematic in such patients [3].

We report here the presence of a rare Hb variant (Hb Hope) that gives an abnormally high HbA1c value when assayed using immunoturbidimetric assay.

Case Report

A 43 year old male patient presented to the surgery OPD with recurrent perianal abscess. On investigation his fasting blood glucose (FBG) was found out to be 310 mg/dl. He was admitted to the endocrinology department of IMS & SUM Hospital and given human insulin with regular monitoring of blood glucose. His serum urea and creatinine level were 22 and 0.7 mg/dl respectively. Serum protein and albumin were 6.5 and 3.7 mg/dl respectively. Urine

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microalbumin was 5.2 mg/l indicating normal renal function in that patient. All other biochemical parameters were within normal range except serum alanine transaminase and aspartate transaminase which were slightly elevated.

His HbA1c% was estimated using “Tina-quant” 2nd generation assay by Cobas Integra 400 plus automated analyser. The HbA1c determination is based on turbidometric inhibition immune-assay (TINA) of haemolysed whole blood samples. This method has been standardised against the approved IFCC (International Federation of Clinical Chemistry and Laboratory Medicine) reference method for the measurement of HbA1c in human blood and can be transferred to results traceable to DCCT/NGSP (Diabetes Control and Complications Trial/National Glycohemoglobin Standardization Programme) by calculations $[HbA1c\% = (HbA1c/Hb) \times 91.5 + 2.5]$ [4]. The result of this patient could not be detected by the instrument, due to very high level of HbA1c which was found out to be >2.75 , although the Hb was within normal range (15.4 g%). When we calculated HbA1c% value using the standardisation according to DCCT/NGSP, the result came out to be >18.8 , which did not correlate with the blood glucose level [estimated average glucose (mg/dl) = $28.7 \times HbA1c\% - 46.7$] [5].

A previous study suggested testing samples for the Hb variants when HbA1c immunoassay values are $>15\%$ [2]. So in search of a Hb variant we performed complete blood count and peripheral blood smear, the results were normal.

Then we analysed Hb by capillary electrophoresis using Minicap Sebia (Tribitron) and to our surprise we found a peak adjacent to HbA which comes in the zone of Hb Hope (Graph. 1).

Discussion

Several commercial methods quantify HbA1c using antibody (Ab) mediated inhibition of latex agglutination or immunoturbidimetric assays.

HbA1c in the sample reacts with anti-HbA1c Ab forming a soluble antigen (Ag)–Ab complex. There is no further complex formation because the HbA1c Ab site is present only once on the HbA1c molecule. Polyhapten react with excess anti HbA1c Ab and the formation of an insoluble Ab-polyhapten complex is turbidimetrically determined. In the hemolysed blood sample, liberated Hb derivative is measured bichromatically and calculations are made [4]. Various previous studies have shown decreased Hb variant interference in the “Tina-quant” 2nd generation immunoassay for HbA1c [6]. Variants that are relatively common are often easily recognised. Laboratories may be alerted to the presence of variant Hbs by results that appear implausible.

Antibodies recognise the N-terminal glycosylated amino acid in the context of 1st 4–10 amino acids of the β chain.

Graph 1 Hb Hope in capillary electrophoresis

These antibodies do not recognise the reversible Schiff base or other glycohemoglobin species including chemically modified derivatives. Hb S and Hb C fall within this susceptible region. Hb F, Hb Graz AND Hb Raleigh are shown to cause decreased HbA1c values by immunoassay [7].

In this case the abnormally high HbA1c raised the suspicion that an abnormal Hb may be present [2]. This led to the further investigations, that identified the variant as Hb Hope (β 136 gly-asp). Hb Hope with a unique electrophoretic property and an undescribed β chain defect was discovered during a survey of Hbs in St.Louis Negroes. The abnormal Hb in the heterozygous state caused neither clinical stigmata nor abnormalities in the RBCs [8].

The increased HbA1c value in this patient may be due to enhanced affinity of the antigen–antibody interaction, thereby leaving less latex-bound antibody to agglutinate with the polyvalent antigen reagent. Other possibilities may be that the glycated forms of Hb variants bind the monoclonal Ab nonspecifically under the conditions of the assay or that the agglutination between unreacted Ab and polyvalent Ag is inhibited by some other mechanisms [9]. Of the less common Hb variants that have been studied, only Hb Himeji (β 140Ala-Asp) has demonstrated falsely increased HbA1c results which were attributed to excessive glycation of Hb Himeji in vivo [10].

This report reminds laboratory workers, biochemists and clinicians, that caution is required when interpreting HbA1c results in individuals with hemoglobinopathies, even when the variant Hb may be of little clinical significance. Technologies like boronate affinity chromatography and electrospray mass spectrometry provide means of accurately determining HbA1c, but results may not reflect the long term glycemic control in conditions affecting formation and turnover of HbA1c in vivo.

The laboratories should have alternative forms of testing such as glycosylated albumin and fructosamine to assist clinician for the determination of glucose control in these individuals.

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